Inactivation of *Escherichia coli* JM109, DH5α, and O157:H7 Suspended in Butterfield's Phosphate Buffer by Gamma Irradiation

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ABSTRACT: Food irradiation is a safe and effective method for inactivation of pathogenic bacteria, including *Escherichia coli* O157:H7, in meat, leafy greens, and complex ready-to-eat foods without affecting food product quality. Determining the radiation dose needed to inactivate *E. coli* O157:H7 in foods and the validation of new irradiation technologies are often performed through inoculation of model systems or food products with cocktails of the target bacterium, or use of single well-characterized isolates. In this study, the radiation resistance of 4 *E. coli* strains, 2 DNA repair deficient strains used for cloning and recombinant DNA technology (JM109 and DH5α) and 2 strains of serotype O157:H7 (C9490 and ATCC 35150), were determined. The *D*-10 values for C9490, ATCC 35150, JM109, and DH5α stationary phase cells suspended in Butterfield's Phosphate Buffer and irradiated at 4 °C were 229 (± 9.00), 257 (± 7.00), 61.2 (± 10.4), and 51.2 (± 8.82) Gy, respectively. The results of this study indicate that the extreme radiation sensitivity of JM109 and DH5α makes them unsuitable for use as surrogate microorganisms for pathogenic *E. coli* in the field of food irradiation research. Use of *E. coli* JM109 and DH5α, which carry mutations of the recA and gyrA genes required for efficient DNA repair and replication, is not appropriate for determination of radiation inactivation kinetics and validation of radiation processing equipment.

Keywords: DH5α, DNA repair, *Escherichia coli*, food irradiation, JM109

Introduction

Treatment of foods with ionizing radiation is a safe and effective method for inactivation of pathogens such as *Escherichia coli* O157:H7 in foods, including meat, poultry, fish, seeds, sprouts, lettuce, and complex ready-to-eat foods (Thayer and others 1995, 2003; Niemira and others 2002; Rajkowski and others 2003; Black and Jaczynski 2006; Sommers and Boyd 2006a, 2006b). In the United States, approvals for the use of irradiation by the U.S. Food and Drug Administration came after extensive review of peer-reviewed scientific studies by government, university, and industry laboratories (Federal Register 2005). Both the radiation and food processing industries use peer-reviewed scientific studies to determine whether to adopt any new technology and to determine the radiation doses required for pathogen inactivation without harming the nutritional and organoleptic qualities of the food product.

Food scientists have used multiple approaches for determining radiation inactivation kinetics for bacterial pathogens such as *E. coli* O157:H7. These include (1) cocktails of the pathogen isolated from food products or associated with a foodborne illness outbreak; (2) use of a single well-characterized isolate for mechanistic studies; and (3) use of nonpathogenic surrogates for use in pilot-plant scale operations or when an appropriate biosafety laboratory is not available. In each of these cases, it is helpful to know the source and genotype of the microorganisms being used. Guidelines, including the identification of factors that influence reproducibility of resistance characteristics for test strains as well as the use of multi-isolate cocktails as opposed to single strains for determination of microbial inactivation kinetics, have been outlined by the Natl. Advisory Committee on Microbiological Criteria for Foods (NACMCF 2006).

In this study, the radiation resistance of 4 *E. coli* strains, suspended in Butterfield's Phosphate Buffer as a model system and irradiated under refrigeration conditions, was examined to evaluate their suitability as nonpathogenic surrogates for use in radiation inactivation studies. *E. coli* JM109 and DH5α carry mutations of the *recA* and *gyrA* genes that are required for DNA repair and replication (Bridges 1971; Sedgwick and Bridges 1972; Mount and others 1975; Kornberg and Baker 1992; Orser and others 1995; Cox 2007; Galletto and Kowalczykowski 2007; Sharan and others 2007). *E. coli* O157:H7 C9490 and 35150, which have been used extensively in the study of food irradiation for determination of inactivation kinetics, were used as comparative controls (Buchanan and others 1998, 1999; Niemira 2005; Sommers and Boyd 2006a, 2006b).

Materials and Methods

E. coli strains

Four *E. coli* strains were utilized in this study. JM109 *recA*1, *endA*1, *gyrA*96, *thi*, *hsdR*17, *supE*44, *rel*A1, Δ(*lac-proAB*)/F' [*traD*36, *proAB*$^+$, *lacI*^{q}, *lacZ*ΔM15] was obtained from Stratagene Inc. (La Jolla, Calif., U.S.A.). DH5α *luxS supE44* Δ*lacU169* (ϕ80d*lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi*-1 *rel*A1 was obtained from Invitrogen Inc. (Carlsbad, Calif., U.S.A.). Both JM109 and DH5α are nonpathogenic *E. coli* K12. *E. coli* O157:H7 strain ENT C9490 was obtained from the Centers for Disease Control and Prevention (Atlanta, Ga., U.S.A.). *E. coli* O157:H7 35150 was obtained from American Type Culture Collection (Manassas, Va., U.S.A.). The *E. coli*

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strains were propagated on tryptic soy agar (37 °C) and stored at 4 °C prior to use.

Strain preparation

Each *E. coli* strain was cultured independently in 30-mL tryptic soy broth (Difco-BRL, Sparks, Md., U.S.A.) in sterile 50-mL conical tubes at 37 °C (200 rpm) for 18 h. The cultures were sedimented by centrifugation (2400 × *g* for 10 min), resuspended in an equal volume of Butterfield's Phosphate Buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.), aliquoted (5 mL) into sterile borosilicate glass tubes, and maintained on ice (30 to 60 min) prior to irradiation. Following irradiation, the samples were serially diluted in BPB using tenfold dilutions, and 1 mL of diluted sample was pour plated using tryptic soy agar (Difco). Three 1-mL aliquots were plated per dilution. The plates were then incubated for 24 h at 37 °C prior to enumeration.

Gamma irradiation

A Lockheed Georgia Co. (Marietta, Ga., U.S.A.) self-contained ^{137}Cs radiation source was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9 × 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The dose rate was 0.085 kGy/min. The temperature during irradiation was maintained at 4.0 (± 1.0) °C by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. The temperature was monitored using 2 thermocouples placed adjacent to the sample tubes. The dose delivered was verified by use of 5-mm alanine pellet dosimeters, which were then measured using a Brucker EMS 104 EPR Analyzer (Billerica, Mass., U.S.A.). Radiation doses used were 0, 0.15, 0.3, and 0.45 kGy.

Statistical analysis

D-10 value is defined as the radiation dose required to achieve a 90% reduction in viable microorganisms. The average CFU/mL of an irradiated sample (N) was divided by the average CFU/mL of the untreated control (N_o) to produce a survivor ratio (N/N_o). *D*-10 value was determined by calculating the reciprocal of the slope provided by the $\log_{10}$ (N/N_o) ratios against irradiation dose. Each experiment was conducted independently 3 times. Statistical analysis was completed using the Descriptive Statistics package of Microsoft Excel (Microsoft Corp. Redmond, Wash., U.S.A.). Graphic presentation was performed using SigmaPlot (SPSS Science, Chicago, Ill., U.S.A.).

Results and Discussion

Previously reported radiation *D*-10 values of *E. coli* O157:H7 suspended in a number of matrices are listed in Table 1. Methodology for determination of radiation *D*-10 value was considered carefully. Buchanan and others (1999) found the *D*-10 values for stationary phase *E. coli* O157:H7 suspended in brain heart infusion broth to be also as low as 50 Gy. Niemira and others (2005) found the *D*-10 values for *E .coli* O157:H7 in BPB to be 180 to 334 Gy. Other factors that affect the radiation resistance of microorganisms include the growth state, with the radiation resistance of *E. coli* O157:H7 and other microorganisms to be significantly lower for actively growing (logarithmic phase) cells against stationary phase cells (Thayer and Boyd 1993; Alpen 1998). The radiation resistance of microorganisms is also inversely related to temperature at the time of irradiation (Thayer and Boyd 1993, 1995, 2001; Sommers and others 2002; Black and Jaczynski 2006; Sommers and Niemira 2007). To reliably calculate *D*-10 values for JM109 and DH5α, which carry multiple mutations in genes

Table 1—Radiation *D*-10 values for *E. coli* O157:H7 obtained using a Cs-137 (0.662 MeV) gamma irradiator in various matrices.

Strains	*D*-10 (Gy)	Medium	Reference
C9490	160 to 240	Apple juice	Buchanan and others (1998)
C9490	100 to 120	Acidified brain heart infusion broth (pH 4.0 to 5.5)	Buchanan and others (1999)
A9124-C1	50 to 70	Acidified brain heart infusion broth (pH 4.0 to 5.5)	Buchanan and others (1999)
B1409	70 to 80	Acidified brain heart infusion broth (pH 4.0 to 5.5)	Buchanan and others (1999)
C9490	119 to 140	Lettuce surfaces (green leaf, boston, red leaf, iceberg)	Niemira and others (2002)
C9490	326 to 339	Lettuce homogenates (green leaf, boston, red leaf)	Niemira and others (2002)
C9490	92	Iceberg lettuce homogenate	Niemira and others (2002)
C9490	180	Butterfield's Phosphate Buffer	Niemira (2005)
C9490-R[a]	99	Butterfield's Phosphate Buffer	Niemira (2005)
35150	334	Butterfield's Phosphate Buffer	Niemira (2005)
35150-R[a]	103	Butterfield's Phosphate Buffer	Niemira (2005)
Cocktail	320 to 390	Beef cheeseburger, veggie burger, hot dog on a bun	Sommers and Boyd (2006a)
Cocktail	410 to 430	Hot dog on a bun under modified atmospheres	Sommers and Boyd (2006b)
Cocktail	290 to 320	Beef, chicken, turkey, pork, lamb	Thayer and others (1995)
Cocktail	390	Ground beef	Thayer and Boyd (2001)
Cocktail	340	Radish sprouts	Rajkowski and others (2003)
Cocktail	1,430	Broccoli seeds	Rajkowski and others (2003)
Cocktail	600	Alfalfa seeds	Thayer and others (2003)

[a]Naladixic acid resistant.

Table 2—Radiation *D*-10 values and log reduction and ratios at 0.45 kGy for *E. coli* O157:H7 strains 35150 and C9490, JM109, and DH5α suspended in Butterfield's Phosphate Buffer.

Strains	*D*-10 value (Gy)	R^2	Log reduction (0.45 kGy)	*D*-10 ratio based on 35150	*D*-10 ratio based on C9490
35150	257 (± 7.00)[a]	0.99	1.80	1.00	ND*
C9490	229 (± 9.00)[a]	0.98	2.03	1.12	1.00
JM109	61.2 (± 10.4)[b]	0.99	7.17	4.20	3.74
DH5α	51.2 (± 8.82)[b]	0.98	> 8.00	5.02	4.47

[a,b]Values with different letters are significant at $P < 0.05$.
*ND = not determined.

required for DNA repair and replication, stationary phase cells were suspended in BPB and irradiated at a temperature of 0 to 4 °C.

The radiation *D*-10 values for *E. coli* C9490, 35150, JM109, and DH5*α* suspended in BPB and maintained at 0 to 4 °C during the irradiation process are listed in Table 2 and graphically represented in Figure 1. The *D*-10 values for *E. coli* O157:H7 35150 and C9490 were 257 (± 7.00) and 229 (± 9.00) Gy, respectively. The log reductions for 35150 and C9490 (Table 2) at a radiation dose of 0.45 kGy were 1.80 and 2.03, respectively. In contrast, the *D*-10 values for JM109 and DH5*α* suspended in BPB were 61.2 (± 10.4) and 51.2 (± 8.82) Gy, respectively, which are statistically significantly ($P < 0.05$) lower than C9490 and 35150. The log reduction for JM109 at a radiation dose of 0.45 kGy was 6.82, while no DH5*α* was recovered at the same dose, indicating an inactivation of > 8.0 log CFU/mL. We were not able to calculate *D*-10 values for exponentially growing JM109 and DH5*α* inoculated on tryptic soy agar plate surfaces and irradiated at ambient temperature due to the extreme radiation sensitivity under those conditions. There was a > 6 log reduction at a dose of 0.15 kGy. Chalise and others (2007) determined the *D*-10 value for JM109 to be < 1 Gy using exponentially growing cells on Luria-Bertani agar surfaces.

The low radiation *D*-10 values and the log reductions for *E. coli* JM109 and DH5*α* in comparison to *E. coli* O157:H7 are not surprising. JM109 and DH5*α* have been extensively altered (*recA*1 and *gyrA*96, and so on) to enhance their usefulness in the cloning and maintenance of high copy number plasmids that contain exogenous DNA. The *recA* gene is required for regulation of the SOS DNA repair response in *E. coli*, and *recA* mutants have impaired ability to repair and recombine their chromosomes following exposure to ionizing radiation, UV light, and chemicals that damage DNA (Bridges 1971; Sedgwick and Bridges 1972; Mount and others 1975; Cox 2007; Galletto and Kowalczykowski 2007; Sharan and others 2007). The *gyrA* gene (encoding DNA gyrase) in *E. coli* is SOS inducible, a mutation of which affects the efficiency of DNA repair and replication (Kornberg and Baker 1992; Orser and others 1995). Naladixic acid resistant mutants (DNA gyrase mutants) of *Salmonella* and *E. coli* are more sensitive to gamma irradiation than their naladixic acid sensitive parent strains when suspended in BPB or on the surfaces of lettuce (Niemira 2005; Niemira and Lonczynski 2006) (Table 1).

The use of *E. coli* isolates such as JM109 and DH5*α* which carry mutations in genes required for DNA repair and replication, which are appropriate in the field of molecular biology and recombinant DNA technology, or mechanistic studies in the field of radiation genetics, are not appropriate for use in the field of food irradiation or validation of irradiation equipment. This study emphasizes the importance of the requisite criteria parameters for establishing the equivalence of alternative methods of food pasteurization as published by the Natl. Advisory Committee on Microbiological Criteria for Foods (2006).

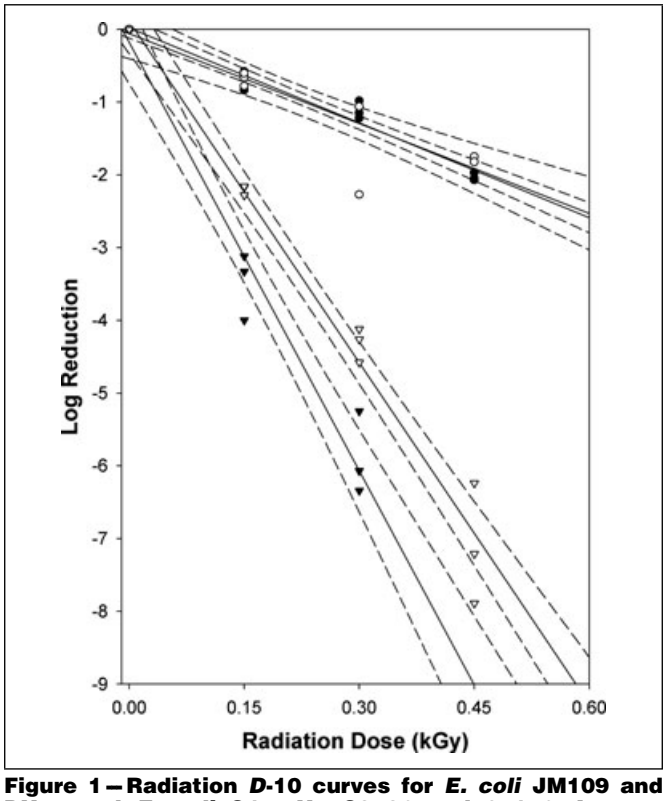

Figure 1—Radiation *D*-10 curves for *E. coli* JM109 and DH5*α* and *E. coli* O157:H7 C9490 and 35150. Log reduction values are shown as symbols: JM109–▽ DH5*α*–▼ C9490–○ 35150–●. Linear regressions are shown as solid lines, while 95% confidence intervals are shown as dashed lines. Each experiment was conducted independently 3 times.

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